



Olfactory response of red palm weevil, *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Dryophthoridae), to host / food volatiles

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Abstract

Red palm weevil, *Rhynchophorus ferrugineus* (Olivier), is a destructive pest of palms across the globe. Concealed nature of the pest is a hurdle in its management. Pheromone traps are a vital component in integrated management of the pest. Previous experiences reveal that use of pheromone, in isolation, trapped lower number of weevils as compared to their use in tandem with food baits. Attempts were made to study physiological and behavioral response of RPW to food baits viz., banana, pineapple and coconut petiole. Electrophysiological responses revealed that head space volatiles trapped from banana elicited the highest EAG response of 0.7 mV followed by pineapple (0.65 mV) and coconut petiole (0.2 mV). Behaviourally, orientation of adult weevils to banana, pineapple and coconut petiole volatiles ranged from 70 to 75 per cent. In field assay, banana volatiles compounds with aggregation pheromone attracted a higher mean number of adult weevils of 55.3, followed by 51.7 with pineapple and 40.6 with coconut petiole volatiles.

Keywords: Behavior assay, EAG, food baits, red palm weevil, wind tunnel, volatiles

Introduction

Red palm weevil (RPW), *Rhynchophorus ferrugineus* (Olivier), also known as coconut weevil or Indian palm weevil (Cox, 1993), is an economically important pest (Murphy and Briscoe, 1999). Originally a major pest of Southern Asia (Faleiro, 2006, Kumara *et al.*, 2015), this weevil has advanced across the globe to more than 90 countries (EPPO, 2014. CABI/EPPO, 2016). RPW is also a significant threat to date palm in the Middle East and Mediterranean basin (Giblin-Davis *et al.*, 2013). In India, RPW is a menace to younger palms, in all the coconut growing states (Faleiro, 2003; Shekar, 2000). Concealed nature of the pest is a major hurdle in detecting the damage caused by them (Murphy and Briscoe, 1999). Indiscriminate and prolonged use of chemical insecticides to manage RPW, poses serious threat to consumers and

environment (Faleiro, 2006). Identification and synthesis of RPW aggregation pheromones, 4-methyl-5-nonanol and 4-methyl-5-nonanone in the ratio of 9:1 (Hallet *et al.*, 1993) was a major breakthrough providing an ecofriendly approach in RPW monitoring and mass trapping (Hallet *et al.*, 1999). Food bait viz., sugar cane bits and split coconut petioles, when used along with pheromone, enhanced attraction of RPW (Faleiro, 2006).

Understanding insect olfaction, including the identification of olfactory receptors, has improved over a period of time (Larsson *et al.*, 2014). In this line, understanding the physiological response of RPW antennae to volatiles of food baits will help us to decipher the weevil's olfactory response that could be exploited for RPW management. The efficacy of food baits (Vacas *et al.*, 2016) trapping density (Vidyasagar *et al.*, 2016) and placement studies (Shukla and Mahmoud, 2018) have been carried out

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in coconut and in date palm gardens. But limited studies have been carried out in India on food volatiles causing the physiological response in RPW that aid in its attraction. Hence, this study investigates the electrophysiological, behavioral and field efficacy of food baits (banana, pineapple, coconut petiole) with aggregation pheromone on the adult RPW.

Materials and methods

Mass culturing of *R. ferrugineus*

Adults of RPW were obtained from infested palms from farmers' garden located in Kasaragod, Kerala (12.4387 °N, 75.2012 °E) and were mass cultured in laboratory for further investigations. RPW colony was maintained on coconut petioles/sugarcane bits. Coconut petioles (8 cm length by 4 cm width) were placed in a polypropylene box (21 x 14 x 13 cm L x W x H) to which five pairs of adult weevils from the collection were released for egg laying (for 24 hrs at 26 ± 0.2 °C and 0L: 24D). The coconut petioles were replaced once in three days. On pupation, the cocoons were placed individually in a plastic container (100 mL) for eclosion. After two generations in the laboratory, the progeny was used for the physiological and behavioral assay.

Trapping of volatile compounds from food baits

The food baits *viz.*, banana, pineapple and coconut petiole (250 g) were placed in Erlenmeyer flask (1000 mL) with 100 mL of water. The volatiles emanating from the fermenting food was acquired using the dynamic head space technique for 6 hrs using a Super Q trap (Supelco). The trapped volatiles were eluted with one mL of dichloromethane (Merck HPLC grade). Multiple trappings were made to collect extracts required for electrophysiological and behavioral assay. All samples were stored at -20 °C until use.

Electrophysiological response of adult RPW

Antennal responses of male and female *R. ferrugineus* adults were recorded by an electro-antennographic system (Syntech, Hilversum, The Netherlands) that consisted of a dual electrode probe, a CS-05 stimulus controller for antennal fixation, and an IDAC 232 box to obtain the results. The antenna was fixed as suggested by

Reinecke *et al.* (2005) with the help of Spectra 360 conductive gel (Parker, Orange, New Jersey). The antenna was subjected to a steady flow of activated charcoal screened air.

Olfactory stimulation system

Ten microliters of the eluted head space volatiles were applied on to filter paper Advantec 5C (110 mm) Japan (3 cm X 5 mm) and was placed into the micro tip pipettes (Tarson 100-1000 µL). This was connected to the stimulus controller by Tygon tube. Firstly, solvent was puffed to the antenna followed by stimulus 60 secs later. Electro-antennogram (EAG) response to head space food volatiles was recorded with three replications per antenna. Antennal response is the number of action potentials, yielded by the stimulation during the time span of one second. Ethyl acetate and aggregation pheromone of RPW was used as reference and clean air puff was used as control. Antennal responses to food volatiles were expressed in mV based on the amplitude of the curve. Corrected EAG responses as described by Dickens (1984) and Visser (1979) were subjected to One-way ANOVA.

Behavioural response of RPW to food volatiles

Wind tunnel experiments were performed to assess the behavioral response of red palm weevil to food volatiles. Purified air was allowed to flow in the tunnel and this was created by suction motor with variable speed. Adult RPW (male and female) were released against the air current. Food volatile (10 µL) loaded on to a filter paper to be evaluated was kept at the end from which the air was let in. The positive response was scored by the number of weevils crossing the midline, taking flight and making source contact. The data was subjected to One-way ANOVA and the means were subjected to Tukeys post hoc test to determine significant difference by SPSS 16.0.

Field evaluation of food baits volatiles with pheromone lure to trap RPW

The field trials were conducted in farmers plot at Chittarikal, Kasaragod district (12.5102 °N, 74.9852 °E), Kerala, India from 2013 to 2014. Prior to initiation of the field trial, a general assessment of the adult weevil population was done. Bucket trap (15 liter) with a window (3 cm X 3 cm) equally spaced from each other was made. The bucket was wrapped with jute bags or coconut fiber and hung by a metal wire on a wooden pole in shade, away from

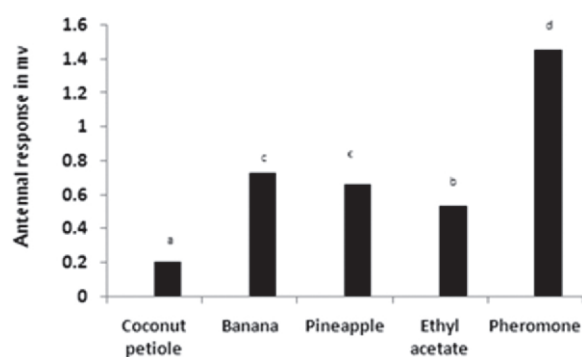
young palms at about 1-2 m from ground. Pheromone 400 mg (4-methyl-5-nonanol and 4-methyl-5 nonanone in the ratio 9:1) sourced from Alfa-Aesar was taken in a polypropylene vial (1.5 mL). This vial was hung in the lid of the bucket. Trapped head space food volatiles (10 mL) were loaded in a polypropylene tube and were hung along with pheromone lure in bucket. Around 1.5 liter of water along with 0.2 mL of imidacloprid was added to the bucket. The water level was replenished every 10 days. The food bait volatiles were replaced every 20 days.

To assess the density of the traps with lure and volatiles of food baits, the traps were placed at the rate of 1 trap per hectare, 2 traps per hectare, 1 trap per 2 hectares and 1 trap per 4 hectares in a randomized block design. The distance between replications was about 500 to 600 m. Mean number of weevils trapped were calculated by One-way ANOVA followed by Tukey HSD test.

Result and discussion

Antennal response of adult RPW to food volatiles

The RPW antennal response to food volatiles (Fig. 1) was measured by the size of amplitude (summated response of neurons in antennae) in EAG. As there was no clear-cut sexual dimorphism in the antennal response between the sexes, a pooled response of the antennae to the individual host volatiles were made.



Bars superscripted by the same letter are not significantly different: $P < 0.05$ by Tukey's HSD test

Fig.1. Antennal response of adult RPW to food volatile

Aggregation pheromone (4 methyl 5 nonanol + 4 methyl 5 nonanone 9:1) caused maximum antennal response of 1.5 mV. Among the food

volatiles, banana and pine apple head space volatiles caused higher antennal response (0.7 – 0.8 mV) and were at par. Coconut petiole caused lowest response of 0.2 mV among the host volatiles. Ethyl acetate, a major constituent of fermenting food bait caused over 0.5 mV. Higher antennal response of RPW to banana and pineapple may be due to presence of esters that has higher number of receptors in the antennae of RPW.

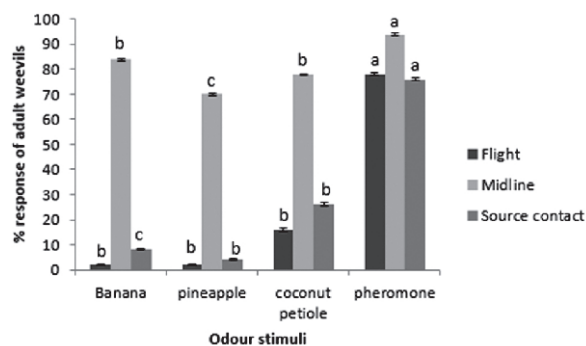
EAG aids in understanding the antennal response of insect to the spectrum of volatiles (Takacs *et al.*, 1997; Karimifar *et al.*, 2011) by way of summated response of neurons (Beck *et al.*, 2012). Volatiles released from hosts are an important signal to cause orientation of insect to find its food and conspecific mate (Bruce and Pickett, 2011). Antennal responses to volatiles, reflects the presence of receptors that are required for attraction or repulsion of insect.

RPW produces aggregation pheromone in response to ethyl acetate released from fermenting food volatiles (Jaffe *et al.*, 1993). Our observation revealed that antennal response in EAG to volatiles trapped from food baits *viz.*, banana and pineapple were higher as compared to ethyl acetate which is also a major palm ester released from food bait (Gries *et al.*, 1994). RPW has been reported to respond to lower concentration of ethyl acetate and ethyl propionate (Guarino *et al.*, 2010). All the compounds released from food baits do not cause physiological or behavioral response in insects but when the blends are perceived in appropriate ratios they act as an attractive host cue for the insect to orient towards them (Bruce and Pickett, 2011). Early fermenting volatile such as ethyl acetate caused higher EAG response in *Rhynchophorus sp.* (Rochat *et al.*, 1993; Gries *et al.*, 1994) Ethyl propionate has also been reported to have caused higher antennal response in *R. ferrugineus* (Guarino *et al.*, 2010). Vacas *et al.* (2014) observed differential EAG response in male and female antennae but our observations did not show any difference in antennal response in male and female counterparts. Blend of plant volatiles from coconut fraction *viz.*, ethyl acetate, ethyl butyrate, phenol, acetaldehyde, propyl acetate acted as kairomones in *R. palmarum* (Rochat and Avand-Faghih, 2000). Ethyl acetate was reported as a kairomone to *R. cuneatus* (Giblin-Davis *et al.*, 1994) and *R. palmarum* (Jaffe *et al.*, 1993). Weevils *viz.*, *Otiorhynchus sulcatus* (F) (Van Tol *et al.*, 2002), *Anthonomus musculus* Say

(Szendrei *et al.*, 2009) has also been reported to be attracted to host produced volatiles.

Behavioural response of RPW to food baits

Compounds that cause physiological response in adult antennae of RPW need not be behaviourally active. Hence, the behavioural response of adult RPW to host volatiles was assessed by behavioural assay in wind tunnel (Fig. 2). Orientation of the weevils to source, flight initiation and source contact to stimuli revealed that all the parameters assessed were high for aggregation pheromone. Among the food baits, the orientations of weevils to banana, pineapple and coconut petiole ranged from 70-75 per cent and were at par. Though coconut petiole volatiles caused lower physiological response in the electro-antennography, their behavioural response was on par with other food baits. This may be attributed to other minor compounds in the trapped volatiles that would have caused lower physiological response but yet essential to initiate the orientation of the weevils to the source.



Bars superscripted by the same letter are not significantly different, $P < 0.05$ by Tukeys HSD test

Fig. 2. Behavioural activity of the adult weevils in wind tunnel to food volatiles

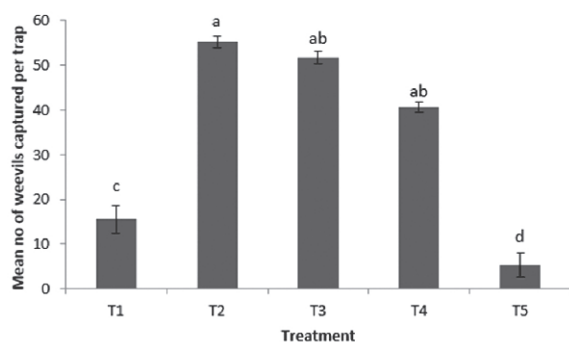
Plant volatiles have an important role in locating host, conspecifics and oviposition sites. Pheromone is a long distant attractant, while odorant blend released from host helps in landing on feeding and oviposition site (Stephens *et al.*, 1999). Since the insect environment is filled with number of volatiles in varying concentration and blends (Bruce *et al.*, 2005), it is important that their receptor

system is highly selective and sensitive (Schröder and Hilker, 2008).

The electrophysiological response was high for fermenting host volatiles of banana and pineapple when compared to coconut volatiles but in case of behavior responses, the weevils oriented more to volatiles from coconut. This may be attributed to the specific cues present in coconut volatiles that are required for insect feeding and oviposition. Receptors of phytophagous insects are perceptive to host plant chemicals that lead them to necessary nutrients (Ruther *et al.*, 2004). Wind tunnel assay has been extensively used to assess the pheromone and kairomone response of insects (Baker and Linn, 1984). As RPW adults are active flyers, wind tunnel assay was best suited to study their flight activity in up wind landing on their bait. We observed that the adult weevils responded to aggregation pheromones in terms of flight initiation and source contact as compared to food volatiles in isolation. Sensitivity and selectivity to host volatiles could vary substantially by the increase in the spectrum of volatiles being emitted from physical damage or due to feeding (Poorjavad *et al.*, 2009).

Efficiency of food volatiles placed with pheromone in trapping RPW

Field trial was laid to assess the effect of food volatiles along with RPW pheromone in farmers plot at Chittarikal, Kasaragod. The mean number of weevils captured per trap was estimated to assess the field efficacy (Fig. 3). When pheromone loaded in polymer membrane @ 400 mg (Alfa Aeser) was used in isolation, over 18 weevils were captured per trap. When pheromone was used in tandem with volatiles of banana, it trapped over 55.3 weevils per trap. Pheromone used in tandem with pineapple and coconut petiole volatiles recorded a weevil catch of 51.7 and 40.6, respectively. When banana volatiles were used in isolation less than 5 weevils were captured per trap.



Bars superscripted by the same letter are not significantly different: $P < 0.05$ by Tukey's HSD test

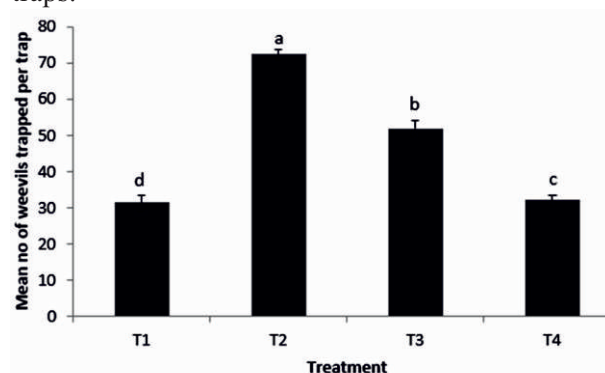
Fig.3. Assessing the food baited pheromone trap
T1-pheromone, T2- pheromone+banana
volatiles, T3-pheromone+pineapple
volatiles, T4-pheromone +coconut petiole
volatiles, T5-banana volatiles

Trapping by baiting with coconut stem tissue has been carried out to manage the weevil populations in India (Abraham and Kurian, 1975) for a long time. Odorants released from a healthy plant are distinctive from the range of odorants discharged from fermented plant tissues (Giblin-Davis *et al.*, 1994; Rochat and Avand-Faghih, 2000). Damage by rhinoceros beetle feeding and wounds caused to palms during the inter-cultivation operations attracts the weevils to lay its eggs. Green coconut petioles, macerated banana, sugarcane bits and molasses were recommended to be used as food baits in the pheromone trap (Oehlschlager *et al.*, 1993, Faleiro *et al.*, 2003). The volatiles emanating from the food baits synergizes the aggregation pheromone (Faleiro and Chellappan, 1999) strongly enhancing the attraction of *Rhynchophorus sp.* to it (Rochat *et al.*, 1993). In field trials addition of water to the food baits, increased the weevil catch, compared to the dry baited trap (Vacas *et al.*, 2014). Our study provides information on using the host volatiles trapped rather than using the food as such that require frequent servicing. Trapped volatiles when used as such helps to do away with the need to change food bait. In addition, the profile of volatile released remains the same as they are not subjected to fermentation.

Field trial to assess the density of traps revealed that placing two traps per hectare captured higher number of weevils per trap (72.6) followed by one trap per hectare (52) (Fig. 4).

Lowest number of weevils was trapped when traps were placed at the rate of one trap per 4 ha. Faleiro *et al.* (2011) has suggested placing four to seven traps per ha, with an infestation higher than one per cent, and one trap per ha in farms with an infestation less than one per cent.

The density of trap for mass trapping of RPW has been reported earlier along with food baits. But the major hurdle in adopting this technology by the farmers is the frequent need to service the trap that is labor and time consuming and increases the cost of operation. The volatiles of food bait when used eliminates the need for frequent servicing of the traps.



Bars superscripted by the same letter are not significantly different: $P < 0.05$ by Tukey's HSD test

Fig.4. Adult weevils trapped at varying density in field trials; T1 – 1 trap per hectare, T2- 2 traps per hectare, T3- 1 trap per 2 hectare, T4-1 trap per 4 hectare

Conclusion

Electro-antennography revealed that volatile in food baits elicits physiological response in the olfactory receptors of adult red palm weevil. In addition to physiological response, they also showed orientation to volatiles from food baits like banana, pineapple and coconut petiole. Using the head space volatiles isolated from food baits increased the trapping efficiency of the pheromone lure when placed at the rate of two traps per ha. As the efficacy of the food bait is dependent on fermenting nature, the quality of the volatiles keeps shifting from the day of their immersion into the bucket traps. Identifying the compounds released in optimum fermenting stage will help to develop blends that would serve as economical and easy to use synthetic food baits. This could be an area of further research in the future.

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